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Rapid bioassay of aluminum toxicity in soil*

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Abstract

Fifty-five acid soil horizons from 19 profiles were evaluated for aluminum toxicity using root elongation as a criterion in a two-day petri dish bioassay. The method proved to be simple, efficient, and precise enough to clearly distinguish aluminum toxicity differences among horizons within and between profiles. Although toxicity patterns within profiles differed, it was common for surface horizons to be less toxic even when very acid. The R^2 for correlations of relative root lengths with pH in H_2O, pH in KCl, soluble and exchangeable aluminum and percent aluminum saturation were only 0.42, 0.45, 0.52, 0.66, and 0.54, respectively, which indicates the need for a bioassay. In a further use of the method, and to demonstrate its efficiency, 243 horizons from 26 profiles were screened. Approximately half of the horizons with a pH of 5.0 or below showed Al toxicity. When used by different operators, with a variety of soil and treatment parameter changes, the two-day bioassay in petri dishes gave consistent rankings of soils by degree of aluminum toxicity.

Introduction

Aluminum concentration can be sufficiently high in acid soils with pH values of 5.5 or below to be toxic to plants. The aluminum species which are responsible for the phytotoxic effect appear to be a small fraction of the total aluminum in the soil solution. An activity of aluminum ions of less than 2 μM in a solution growth medium for wheat and sorghum (Hill *et al.*, 1969; Parker *et al.*, 1988) and less than 1 μM for red clover (Kinraide *et al.*, 1985) caused reduction in plant root growth. Blamey *et al.* (1983) had major reductions in soybean root elongation from 5 or 10 μM total aluminum in acid nutrient solutions with an 80% reduction when the activity of aluminum monomers approached 20 μM. Yet, total aluminum in soil solutions from samples studied in our USDA-ARS lab have run higher than 300 μM without producing any appreciable phytotoxic effects. Adams and Hathcock (1984) also noted ambiguities between solution aluminum content and phytotoxicity when the 15-sec. 8-hydroxyquinoline R. method was used to measure a more active aluminum fraction. Aluminum toxicity appeared in some soils with 0.4 μM aluminum in soil solution, but not in others, and did not appear in one soil with 11.5 μM aluminum in soil solution. Calculated aluminum activities ranged from 0.3 to 7.8 μM, respectively.

It is important to know which aluminum species in the soil are responsible for the toxicity, and to understand plant response to aluminum. Present methods of determining aluminum speciation are, however, only operationally defined and, due to the complexity of the soil-solution system, are, at best, only approximate. Moreover, it is not possible at present to run routine chemical tests to accurately determine the level of toxic aluminum species in

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individual soils. Thus, an efficient plant bioassay system for identifying Al-toxic soils is important now, and for the foreseeable future.

Plant bioassay in growth chamber, greenhouse or field experiments can assess the phytotoxic effect of soil aluminum (Adams and Hathcock, 1984; Wright *et al.*, 1987). The bioassay for identifying Al-toxic soils can be simplified based on the observation that aluminum toxicity first affects the roots. Basic assumptions are made that root growth reduction is due to a specific form or forms of aluminum which are difficult to measure chemically, and that the bioassay provides a measure of aluminum phytotoxicity. It is possible to use root length to quantify aluminum toxicity in soil (Karr *et al.*, 1983). At this early growth stage, in many species the root system is limited to one primary root, which simplifies the measurement. Hill *et al.* (1989) showed that the tolerance or sensitivity of sorghum (*Sorghum bicolor* L. Moench) to aluminum was demonstrable in 48 hours by root elongation of germinated seeds and that either a solution culture containing only aluminum and calcium salts or selected soils with a high percentage of aluminum saturation evaluated tolerance equally well. Roots affected by aluminum lack root hairs and are thickened, brown, short and multi-branched. During the first days of growth the major root symptoms are a lack of root hairs and inhibition of elongation. Calcium deficiency is another common cause of reduced root length and occurs especially in highly weathered tropical soils. Ritchey *et al.* (1983) have used a rapid system of bioassay to study calcium deficiency in Oxisols of the Cerrados of Brazil and have adapted the system to screen simultaneously for calcium deficiency and aluminum toxicity (Ritchey *et al.*, 1986).

For a bioassay to serve as a routine test it should be simple and rapid to conduct. Karr *et al.* (1983) and Ritchey *et al.* (1983, 1986) have presented rapid root bioassay methods for identifying Al-toxic soils. Ritchey's technique uses soil in small plastic cups and natural or artificial light with root elongation of sprouted seeds measured after four days. Karr's procedure uses soil in petri dishes with no light and root length is measured after two days. Both are based on relative root length is an unknown soil to that in a check soil which has no aluminum toxicity and no calcium deficiency.

The hypotheses of this study are that (i) root length can be used as a criterion to identify Al toxic soils and (ii) root length measured two days after germinated seeds have been placed in the soil is as good as roots grown for longer periods.

Materials and methods

Soils to be tested for aluminum toxicity were passed through a 2 mm sieve and then wetted to approximate a 33 KPa moisture potential by spraying with deionized water while hand mixing. The moist soil was equilibrated for at least 12 hours. Wheat (*Triticum aestivum*, L. cv. Abe, Hart, Wampum and Yeccora Rojo) and sorghum (*Sorghum bicolor*, L. cv. SC-283 and TAM 428) were used as indicator plants. Seeds were placed on moist germination paper and at the end of a 36–48 hour germination period uniformly sprouted seeds were selected for planting.

For the two-day test, plastic petri dishes (9.0 × 9.0 × 1.5 cm) were filled with soil and planted along one edge with 5 or 10 sprouted seeds. The lids were taped onto the dishes which were held upright, rapped gently on the bench to assure good root–soil contact, placed vertically in a pan and enclosed in a plastic bag to reduce moisture loss. After incubating in the dark for two days at 25°C, the seedlings were removed and, depending on the experiment, the longest or total root length was measured on each plant. In the four-day technique five sprouted seeds were planted in moist soil in 200 ml plastic cups and replicated four times. The cups were enclosed in a plastic covered box containing wet paper towels to retain humidity and placed in a growth chamber for four days.

To establish the ability of the two-day test to differentiate degrees of aluminum toxicity with precision, soils from Southern Indiana (Table 1) were selected to include several major soil series and yet be acid enough that most horizons could show aluminum toxicity. Six of the soils were Alfisols and six were Ultisols. The indicator plant was aluminum sensitive Abe wheat planted at a rate of five seedlings per petri dish with six replications per horizon. Root growth was compared to pH measured in water and in 1 *M* KCl (1:1), to exchangeable aluminum, % aluminum saturation and soluble aluminum. Exchangeable H and Al were determined in a KCl extract by the method of

Table 1. Selected characteristics of the soils used to evaluate the two-day petri dish bioassay and relative root lengths (RRL) measured with the bioassay

Horizon[a]		Aluminum			pH		RRL[b]
		Exch. cmol(+)kg^{-1}	Satn %	Soln μM	H_2O	KCl	%
Avonburg (*Typic Hapludalfs*)							
A1	(0–8)	0.1	3	26	4.7	4.1	86
E	(8–11)	2.3	45	62	4.1	3.6	86
Bt1	(11–15)	5.8	51	148	3.9	3.3	47
Bt2	(15–21)	8.9	59	185	3.9	3.2	41
Bx	(21–28)	8.2	52	185	3.9	3.1	35
Bloomfield (*Psammentic Hapludalfs*)							
Ap	(0–8)	1.0	65	95	4.3	4.0	90
B	(8–20)	0.7	23	10	5.0	4.4	100
Caneyville (*Typic Hapludalfs*)							
A	(0–5)	1.0	65	95	4.3	3.4	81
Bt1	(5–16)	4.7	21	13	4.5	3.3	74
Carmel (*Typic Hapludalfs*)							
Ap	(0–6)	3.2	48	143	4.6	3.5	76
Bt1	(6–12)	7.1	52	111	4.6	3.4	50
Bt2	(12–17)	8.1	56	98	4.6	3.3	47
Clermont (*Typic Ochraqualfs*)							
Ap1	(0–4	0.2	1	13	6.7	6.4	83
Ap2	(4–13)	0.1	1	6	6.9	6.5	93
E	(13–21)	2.0	21	24	4.5	3.4	88
Bt1	(21–30)	3.1	27	52	4.4	3.3	75
Frederick (*Typic Paleudults*)							
A	(0–1)	0.6	5	81	4.8	4.4	68
E	(1–7)	3.6	80	26	4.1	3.6	47
Bt1	(7–14)	4.8	85	48	4.2	3.5	41
Gilpin (*Typic Hapludults*)							
Ap	(0–12)	2.5	68	60	4.5	3.7	100
BE	(12–22)	6.5	79	259	4.3	3.5	50
Bt	(22–46)	7.1	73	296	4.3	3.5	49
C	(46+)	5.1	72	126	4.3	3.6	70
Grayford (*Typic Paleudults*)							
Ap	(0–3)	0.5	9	13	4.4	3.6	94
E	(3–9)	1.3	31	17	4.4	3.5	82
BE	(9–13)	1.5	27	19	4.6	3.5	98
Bt1	(13–20)	5.5	46	81	4.6	3.3	61
Pekin (*Aquic Fragiudults*)							
AP	(0–8)	1.0	19	31	4.5	3.8	86
Bt1	(8–15)	1.2	13	13	4.7	3.9	73
Peoga[1] (*Typic Ochraqualfs*)							
Ap	(0–8)	0.2	2	7	6.6	5.6	100
BEg	(8–18)	2.2	30	89	4.7	3.5	80
Btg	(18–28)	3.1	52	154	4.2	3.2	53
Peoga[2] (*Typic Ochraqualfs*)							
Ap	(0–8)	0.1	2	8	5.4	4.8	100
BEg	(8–18)	1.6	30	24	4.7	4.0	95
Bg	(18–28)	2.8	46	81	4.3	3.8	64
Peoga[3] (*Typic Ochraqualfs*)							
AP	(0–8)	0.1	1	12	6.7	6.4	91
BEg	(8–18)	0.6	8	6	5.4	4.5	80
Peoga[4] (*Typic Ochraqualfs*)							
A	(0–8)	2.4	50	27	4.3	3.8	53
BEg	(8–18)	2.9	78	289	4.3	3.7	42
Rockcastle[1] (*Typic Hapludults*)							
A	(0–2)	3.0	33	41	4.6	3.7	92
E	(2–10)	3.8	49	148	4.6	3.7	82
Bt	(10–20)	4.6	52	167	4.7	3.8	65
BC	(20–30)	3.2	40	63	4.7	3.8	72
Rockcastle[2] (*Typic Hapludults*)							
A	(0–8)	4.9	90	109	4.2	3.3	45
BE	(8–18)	5.4	87	220	4.4	3.3	42
Bt	(18–25)	6.6	84	326	4.3	3.2	39
Zanesville[1] (*Typic Fragiudults*)							
Ap	(0–6)	3.5	51	98	4.5	3.4	77
Bt	(6–25)	7.4	66	144	4.5	3.2	38
Bx	(25–42)	6.2	62	167	4.5	3.1	44
Zanesville[2] (*Typic Fragiudults*)							
Ap	(0–6)	2.6	25	37	4.7	3.8	83
Bt	(6–25)	6.5	59	161	4.5	3.7	61
Zanesville[3] (*Typic Fragiudults*)							
Ap	(0–6)	0.2	3	9	5.4	4.4	91
Bt	(6–25)	3.9	40	44	4.7	3.9	78
Zanesville[4] (*Typic Fragiudults*)							
Ap	(0–6)	0.2	2	12	6.4	5.9	100
Bt	(6–25)	3.5	43	56	4.6	3.9	80

[a] Horizon depth in cm.

[b] Relative to root length in a limed Peoga A horizon.

Yuan (1959). The % Al saturation was calculated using the cation exchange capacity derived by summation of acidity with Ca, Mg, K, and Na extracted by neutral, molar ammonium acetate (Thomas, 1982). The soluble aluminum was measured colorimetrically with Aluminon in solution extracted from a saturated paste made with 0.01 *M* $CaCl_2$ solution. Regression analyses were made with the chemical measurement as the independent variable and the relative root length as the dependent variable.

Subsequently the ability of the petri dish bioas-

Table 2. Chemical characteristics of ten soil horizons used for evaluation of the rapid bioassay for Al toxicity

Soil	pH		Exch. cations			Cation Exch. Satn.			Soln Al
	H_2O	$CaCl_2$	CEC	Ca	Al	Bases	Ca	Al	μM
			(cmol (+) kg^{-1})				%		
Gilpin BA	5.2	4.6	4.8	1.7	1.2	58	36	25	10
Dekalb A	4.2	3.8	6.3	3.2	0.7	67	51	12	392
Ashe Bw	4.9	4.4	0.7	< 0.1	0.4	18	5	55	20
Dunmore A	4.4	3.7	4.4	2.3	0.2	78	51	5	380
Dandridge E	4.6	4.2	6.1	2.7	1.5	56	44	24	142
Berks A	4.0	3.5	8.8	2.6	3.8	42	29	43	599
Tate BA	4.5	4.2	6.2	0.8	3.7	23	13	60	41
Dekalb Bw	4.9	4.2	2.0	0.1	1.0	17	6	50	91
Porters Bw	4.7	4.2	4.0	0.1	3.2	4	1	79	273
Lily Bt	4.6	4.1	3.3	0.1	2.4	7	2	74	71

say to efficiently screen a large number of acid soils was tested. A single petri dish of each soil was planted with 10 sprouted seeds of Abe wheat and root growth was compared to a check soil, *i.e.*, limed Peoga A horizon. This study included 243 horizons from 46 profiles representing 34 soil series from 15 counties. They were mostly from southern Indiana, a region of older soils, and each had at least one horizon with soil in the 3.8 to 5.5 pH range.

Finally, ten acid soil horizons from the Appalachian region with pH 4.0 to 5.2 (1:1 soil–water ratio) were selected for their diverse chemical and physical characteristics (Table 2). Nine bioassays studies run on these soils were compared to test the hypotheses that (i) two days of growth would be as effective as four days in detecting Al toxicity and (ii) the rapid bioassays would give comparable results when conducted by different operators using as test plants various varieties of wheat or sorghum exhibiting a range of tolerance to aluminum. Several assays also included addition of 0.025 cmol Ca kg^{-1} of soil to eliminate the possibility of calcium deficiency. Correlations were made between the relative root lengths by the various bioassay runs as well as between the mean relative root lengths and several easily measured chemical parameters of the soils. Solution aluminum in this study was measured by inductively coupled plasma on solution obtained by centrifugation of equilibrated, moist soil. pH was measured in 0.01 *M* $CaCl_2$ (1:1). Other measurements were made as in the first study above.

Results and discussion

The petri dish bioassay clearly identified which horizons have toxic levels of aluminum and differences between horizons. Figure 1 shows typical

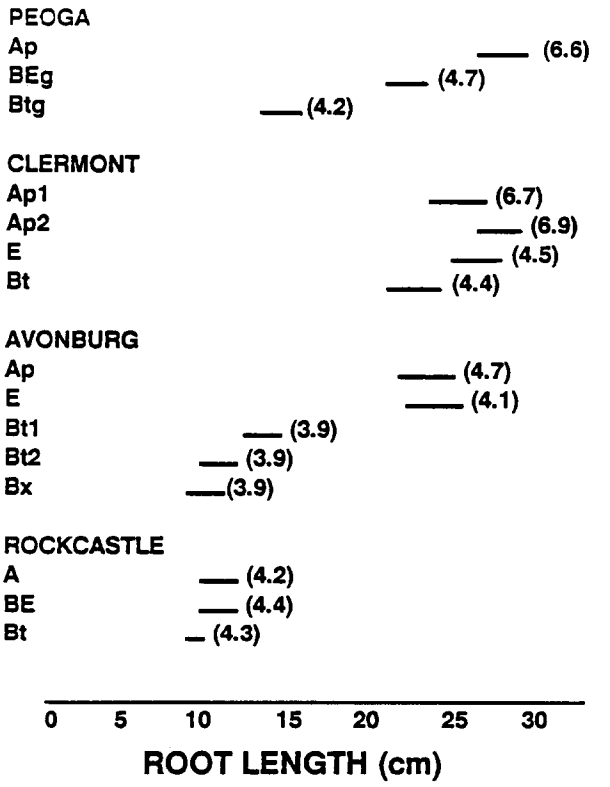

Fig. 1. Mean root length of the Abe wheat in petri dish bioassays of sequential horizons in four soils. The horizon pH is given in parenthesis. The bar represents the 95% confidence interval for the populat ean of six trials.

root lengths and their 95% confidence intervals. The confidence intervals are narrow enough to clearly separate horizons of differing toxicities. Root lengths in a cultivated, limed Peoga (Table 1, Peoga (1)) were 100, 80 and 53% of the root length of the nontoxic check soil in the Ap, BEg and Btg horizons, respectively. By contrast, an untilled Peoga from an adjacent woods (Table 1, Peoga (4)) was 53 and 41% of check for its Al and BEg horizons. The Peoga is an Alfisol developed in silty outwash of the late Pleistocene glacial epoch.

The other soils of Figure 1 illustrate the inconsistent occurrence of aluminum toxicity. The Clermont soil, an Alfisol in Wisconsinian loess, shows that acid soils are not necessarily aluminum toxic as its E and Bt horizons at pH 4.5 and 4.4 have little or no toxicity. The Avonburg soil on the same landscape demonstrates again that some acid soil horizons are not aluminum-toxic and shows the fairly common pattern of acid surface horizons being nontoxic or less toxic than subsoil horizons. In contrast, the Rockcastle (2), an Ultisol formed in grey shales, shows that soils can have severe aluminum toxicity even at the surface.

There is a tendency for the RRL to be lower in the B horizons (Table 1). This toxic zone can be quite near the surface as all B horizons in this study began between 12 to 30 cm below the surface. Ritchey *et al.* (1988) demonstrated that shallowness to aluminum toxic layers can seriously limit the ability of a sensitive species to extract soil moisture. Also because most nutrients are obtained from the A horizon, soils with toxic surface horizons such as the Peoga (4) and the Rockcastle (2) of Table 1 which reduce root length and root hairs of sensitive species could diminish uptake of nutrients. This could be critical for nutrients which are in low concentration and dependent on diffusion for transport to nearby roots.

It is obvious that soil pH measured in water is not a valid predictor of the presence of aluminum toxicity when one notes in Table 1 that soils with pH values near 4.5, such as Gilpin Ap, the first three horizons of the Grayford and the Rockcastle (1) A are all nontoxic while in other horizons with comparable pH values (*e.g.* Zanesville (1) Bt) root growth is reduced to as little as 38% of the check. Correlations of relative root lengths to pH in water and in KCl for the horizons of the different soil profiles gave R^2 values of 0.42* and 0.45**, respectively. Aluminum toxicity seldom occurs above pH 5, and 47 of the 55 horizons in this study were pH 5 (in water) or below. The R^2 values for correlation of soluble aluminum, exchangeable aluminum and percentage aluminum saturation to root elongation were 0.52**, 0.66** and 0.54**, respectively. While these correlations are statistically significant, the ability to identify individual Al toxic soils based solely on pH, exchangeable aluminum, % aluminum saturation or soluble aluminum is poor. This indicates a need for a simple bioassay to screen soils for aluminum toxicity.

The petri dish bioassay makes it possible to efficiently evaluate large numbers of soils for aluminum toxicity. One person can prepare five to ten soils per hour on day one. Planting, on day three, and measuring root length, on day five, also takes about one hour for each five to ten samples. Thus, the bioassay was convenient for a study using a single petri dish of soil from each of 243 horizons of 46 acid soil profiles from Indiana (data not shown). Little or no toxicity (root elongation reduced by less than 20%) occurred in any horizon of 17 profiles while 29 profiles had horizons causing from 20 to 76% reduction in root elongation during the two day growth period. Of the 29 profiles with toxicity, 3 had horizons which reduced root elongation by over 60%, 15 had horizons giving 40 to 60% reduction and 11 had horizons giving 20 to 40% reduction. Of the 113 horizons with a pH of 5.0 or below, only 50 produced phytotoxic effects. Thus mineral soils which are sufficiently acid to maintain toxic levels of aluminum in their solution are not always phytotoxic.

For an efficient screening method there is an advantage in a bioassay that requires neither special growing conditions nor a long growth period. While the four-day method of Ritchey *et al.* (1983) appears to function well, the four days of growth may be more time than is necessary. In the petri dish technique the two-day root growth is only dependent on energy and nutrients supplied by the seed plus water and Ca supplied by the soil solution. Also, the ambient temperature in the lab is satisfactory since measurement is relative to a check soil. Light is not needed during the two days of the test. Thus various modifications of the bioassay procedure were compared.

Table 3 gives data from two-day and four-day

Table 3. Relative root lengths (%) from rapid bioassays for Al toxicity of ten soil horizons; conducted with varied parameters (operators, time, cultivars, calcium)

Soil	RRL				
	I[a]	II[b]	III[c]	IV[d]	V[e]
Non-toxic					
Gilpin BA	100	87	100	84	96
Dekalb A	99	78	89	100	85
Ashe Bw	95	100	93	98	85
Dunmore A	93	78	89	77	79
Dandridge E	90	87	85	95	92
Moderately toxic					
Berks A	46	53	18	23	54
Tate BA	26	51	44	44	56
Dekalb Bw	26	59	40	34	56
Very toxic					
Porters Bw	18	23	23	24	27
Lily Bt	18	29	24	19	29
Mean RRL	61	65	61	60	66

[a] Hart wheat, 4-day growth chamber test, operator 1.
[b] Hart wheat, 4-day growth chamber test, operator 2, 0.025 cmol Ca kg^{-1} added to all soils.
[c] Hart wheat, 2-day petri dish test, operator 3.
[d] TAM 428 sorghum, 2-day petri dish test, operator 3.
[e] Mean of 9 test runs using combinations of 3 different operators, 2 Al-sensitive wheats (Hart, Wampum) 1 Al-tolerant wheat (Yecorro Rojo), 1 Al-sensitive sorghum (TAM 428), 1 Al-tolerant sorghum (SC 283), and 3 growing systems (2- and 4-day petri dish and 4-day growth chamber).

tests conducted under a variety of conditions. The two-day RRL (columns III and IV) appear comparable to the four-day values (columns I and II). Tests I and II were each run by a different operator with III and IV by yet a third person. The screening in tests I, II, and III were with aluminum-sensitive Hart wheat and IV was with sensitive TAM 428 sorghum. Our experience in screening both wheat and sorghum cultivars indicated that they have somewhat comparable levels and ranges of tolerance to aluminum. Thus, both Hart wheat and the TAM 428 sorghum worked equally well as indicator plants.

The mean values in column V include tests I to IV plus five additional tests, one each with sensitive Wampum and Hart wheat, two with tolerant Yeccora Rojo wheat and one with SC 283, a tolerant sorghum. The tolerant varieties result in a mean % RRL for the toxic soils (Berks A, Tate BA, Dekalb Bw, Porter Bw, and Lily Bt) slightly greater than in tests I and IV. Also a very small addition of Ca as $Ca(OH)_2$ to all soils in three of the tests to assure adequate Ca for root growth resulted in an apparent small improvement in growth in the toxic soils. This effect is seen in Test II which is one of the three tests which received the added $Ca(OH)_2$ and is included in the mean, V. However, the absence of additional Ca did not affect the ability of the plants to detect the aluminum toxicity. Thus, while confounding does not permit statistical comparison of the results, all modifications of the test gave comparable assays of the soils into nontoxic, moderately toxic, and very toxic categories.

The most erratic soil was the Berks A. The problem appeared to be attributable to its physical character, a mixture of coarse organic matter and finer sandy mineral matter such that the composition of the sample in which the roots grew depended on how it was sieved and handled.

From our experience with these soils, we would classify the first five (Gilpin Ba, Dekalb A, Ashe Bw, Dunmore A and Dandridge E) as nontoxic, the next three (Berks A, Tate Ba and Dekalb Bw) as moderately toxic, and the last two (Porter Bw and Lily Bt) as very toxic to species or varieties of plants which are sensitive to aluminum.

While no detailed study has been made of precision, replicates compared well. The coefficient of variation of the 10 plants within a petri dish in the two-day bioassay is typically less than 25%. The main source of variation is seedling vigor. Coefficients of variation can be reduced by obtaining high quality seed, germinating an excess number of seeds and then carefully selecting for those that show uniform germination and initial growth.

Comparison of chemical parameters of the 10 Appalachian soil horizons (Table 2) with root growth (Table 3) illustrates the present difficulty of finding an easily measureable chemical parameter which will predict aluminum toxicity. The R^2 from the correlation of mean relative root lengths with percent aluminum saturation, percent calcium saturation, and exchangeable aluminum are 0.66*, 0.50*, and 0.44*, respectively. The pH in H_2O and $CaCl_2$ and the total aluminum in the soil solution gave nonsignificant correlations. Thus, in this group of acid soil horizons, selected for their diverse properties and high probability of exhibiting aluminum toxicity, pH and solution aluminum relate poorly with inhibition of root elongation. While the correlation of percentage

aluminum saturation with RRL was highly significant, it would not be able to accurately predict the presence of toxic levels of aluminum in individual soils. Adams and Hathcock (1984) also demonstrated a poor relationship between percentage Al saturation and Al toxicity. Hue *et al.* (1986) suggested that the presence of nontoxic Al-organic complexes may account for the poor relationship between solution aluminum and root growth. However, because we can not easily determine or speciate the Al-organic complexes the bioassay holds the most promise for identifying aluminum toxic soils.

Conclusions

The 2-day petri dish bioassay is very effective in identifying aluminum toxic soils. The bioassay directly measures the physiological sensitivity of a plant to soil aluminum by the root growth inhibition of aluminum-sensitive species in systems where other inhibiting factors are absent. This occurs with minimal interference of other growth factors when the root of a recently germinated seed is measured after only two days in a soil. The bioassay is very efficient, each of the three stages (preparing the soil, planting and measuring root lengths) requires about one hour per 10 soils. The system requires no additional attention.

Many soil horizons with pH values of 5 or below were not toxic to plant roots. The poor relationship between pH and root length indicates that we cannot assume that poor plant growth on an acid soil is the result of aluminum toxicity. Even soils with high levels of soil solution aluminum may not produce toxic effects in plants. Much of the solution aluminum in non-toxic, acid soils must be in complexes or other forms which keep the activity of toxic forms very low.

The consistency of results when test parameters are varied indicate that the two-day petri dish technique should be a useful routine test or diagnostic tool for work with acid soils.

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References

Adams F and Hatchcock P J 1984 Aluminum toxicity and calcium deficiency in acid subsoil horizons of two coastal plants soil series. Soil Sci. Soc. Am. J. 48, 1305–1309.

Blamey F P C, Edwards D G and Asher C J 1983 Effects of aluminum OH:Al molar ratios, and ionic strength on soybean root elongation in solution culture. Soil Sci. 136, 197–207.

Hill P R, Ahlrichs J L, Ejeta G 1989 Rapid evaluations of sorghum for aluminum tolerance. Plant and Soil 114, 85–90.

Hue N V, Craddock G R and Adams F 1986 Effect of organic acids on aluminum toxicity in subsoils. Soil Sci. Soc. Am. J. 50, 28–34.

Karr M C 1985 Applying the petri dish bioassay to the characterization of aluminum toxicity and related chemical parameters. MS Thesis, Purdue University, 126 pp.

Karr M C, Coutinho J and Ahlrichs JL 1983 Determination of aluminum toxicity in Indiana soils by petri dish bioassay. Proc. Indiana Acad. Sci. 93, 405–411.

Kinraide T B, Arnold R C and Baligar V C 1985 A rapid assay for aluminum phytotoxicity at submicromolar concentrations. Physiol. Plant. 65, 2245–2250.

Parker D R, Kinraide T B and Zelazny L W 1988 Aluminum speciation and phytotoxicity in dilute hydroxy-aluminum solutions. Soil Sci. Soc. Am. J. 52, 438–444.

Ritchey K D, Sousa D M G, Rodriguez G E, Wright R J and Baligar V C 1986 Agron. Abstr. p. 213.

Ritchey K D, Silva J E and Sousa D M G 1983 Relacao entre teor de calcio no solo e desenvolvimento de raises avaliado por um metodo biologico. R. Bras. Ci Solo. 7, 269–275.

Ritchey K D, Baligar V C and Wright R J 1988 Wheat seedling responses to soil acidity and implications for subsoil rooting. Comm. Soil Sci. Plant Anal. 19, 1285–1293.

Thomas G W 1982 Exchangeable cation. *In* Methods of Soil Analysis. Part 2. Ed. AL Page. pp. 159–165. Am. Soc. Agron., Madison, WI.

Wright RJ, Baligar VC and Wright S F 1987 The influence of acid soil factors on the growth of snapbeans in major Appalachian soils. Comm. Soil Sci. Plant Anal. 18, 1235–1252.

Yuan TL 1959 Determination of exchangeable hydrogen in soils by a titration method. Soil Sci. 88, 164–167.